

Applicant : Myra A. Lipes et al.
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Attorney's Docket No.: 10276-015002

REMARKS

Applicants hereby submit that the enclosures fulfill the requirements under 37 C.F.R. §1.821-1.825. The amendments in the specification merely insert sequence identifiers in the specification and replace the original paper copy of the Sequence Listing with a substitute Sequence Listing which contains all the sequence disclosures of the instant application. I hereby state, as required by 37 C.F.R. §1.821(g), that the enclosed submission includes no new matter.

Attached hereto is a marked-up version of the changes made to the specification by the current amendment.

Please apply any charges or credits to Deposit Account No. 06-1050.

Respectfully submitted,

Date: 7/10/01

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Version With Markings to Show Changes Made

In the specification:

Paragraph beginning at page 11, line 25 has been amended as follows:

-- Figure 2 is a schematic diagram depicting the construction of the rat proopiomelanocortin (POMC)/mouse preproinsulin II (POMC-Ins) fusion transgene. P = preptide coding region; B = B-chain coding region; C = C-peptide coding region; A = A-chain coding region; exons 1-3 (E1-3) are as indicated. Segments of Primer 3 (SEQ ID NO: 4), PPI-2 (SEQ ID NO: 5), and Primer 1 (SEQ ID NO: 6) are also shown.--

Paragraph beginning at page 20, line 16 has been amended as follows:

--The POMC-Insulin transgene consisted of the POMC promoter region linked to the structural region of the mouse preproinsulin II (Ins) gene (Fig 2). To excise the 5' regulatory region of the Ins gene yet preserve the translation initiation start site at position 1132, a novel Hind III restriction site was created at position 985 by site-directed mutagenesis using the recombination polymerase chain reaction (PCR) technique (Jones, D.H., Sakamoto, K., Vorce, R.L. & Howard, B.H. (1990) *Nature (London)* **344**, 793-794). A 2.4 Kb genomic Bam HI Ins fragment (Wentworth, B.M., Schaefer, I.M., Villa-Komaroff, L. & Chirgwin, J.M. (1986) *J. Mol. Evol.* **23**, 305-312) was cloned into pBluescript (pBS, Stratagene). The recombinant Ins-pBS vector was linearized in two separate restriction enzyme digestion reactions with Bal I (position 846) and PfiM I (position 1237). These templates were then amplified in two separate PCR reactions using primer 3: 5'-*CAATCAAAGCTTCAGCAAGCAGGAAGGTAC*-3' (SEQ ID NO:1) (corresponding to sense nucleotides 977-1008, mutagenesis sites underlined, region of complementarity to primer 3 in italic) and primer 2: 5'- TCG TGT AGA TAA CTA CGA TAC G -3' (SEQ ID NO: 3), corresponding to nucleotides 2050-2071 of pBS. The PfiM I template was amplified with primer 1: 5'-*GCTGAAGCTTTGATTGTAGCGGATCACTAG* -3' (SEQ ID NO:2) (corresponding to antisense nucleotides 994-962, mutagenesis sites underlined, region of complementarity to primer 1 in italic) and primer 4 (the entire primer 4 was complementary to primer 2). The PCR products were mixed together and cotransfected into bacteria. The Bal

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I/PfiM I fragment of a plasmid containing the Hind III mutation was then ligated into Ins-pBS that had not undergone PCR amplification. DNA sequencing of the PCR-amplified Hind III/PfiM I region did not reveal any cloning artifacts or polymerase errors.--